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INCREASED EXPRESSION OF ARTHRITIC MARKER GENES IN THE CARTILAGE OF SIRT1 NULL MICE

O.H. Gabay[1], E. Lee[1], R. Booth[1], V. Gagarina[1], M. Dvir-Ginzberg[2], D.J. Hall[1]
[1]NIH, Bethesda, MD; [2]2Lab. of Cartilage Biology, Inst. of Dental Sci., Faculty of Dental Med., The Hebrew Univ. Hadassah Ein Kerem, Jerusalem, Israel

Purpose: Osteoarthritis (OA), the most frequent age related disease present in the west, is a multi-factorial imbalance between cartilage anabolism and catabolism. To understand the mechanisms underlying OA, we focused on the protein deacetylase SirT1, a factor known to prolong organism lifespan. SirT1 has been previously shown to regulate apoptosis and cartilage-specific gene expression in human chondrocytes. Recent data also indicates that SirT1 is a potent inhibitor of the matrix metalloproteinases (MMPs). MMPs are the most well known of arthritis marker genes that play a central role in cartilage degeneration. In order to evaluate the role of SirT1 in cartilage homeostasis, we assessed MMP and ADAMTS expression in the cartilage of SirT1 null mice.
Method: We used SirT1 Wild-type (WT) and SirT1 Null mice in the analysis. The SirT1 Null mice do not express SirT1. Articular cartilage was harvested from hind paws in 1 to 3 week old mice. The cartilage was processed for both immunohistochemistry and subculture of chondrocytes.
Results: Articular cartilage tissue sections from SirT1 Null mice exhibited low levels of type 2 collagen, aggrecan and glycosaminoglycans (GAG) compared to SirT1 WT mice at 1 week or 3 weeks. Protein levels of aggrecan and type 2 collagen were also decreased in the chondrocytes derived from SirT1 Null mice. In contrast, protein levels of MMP-3, MMP-8, MMP-9 and MMP-13 were elevated in the SirT1 Null mice compared to WT. Finally, DBC1 (deleted in breast carcinoma 1), a known Sirt1 associated protein that represses SirT1 enzymatic activity, was found to be elevated in the SirT1 Null mice compared to WT.
Conclusion: The data from this animal model indicate that SirT1 is a negative regulator of the genes responsible for cartilage degradation in arthritis; namely the MMPs and DBC1. The role of SirT1 as a putative anti-arthritic gene is consistent with its general function as an anti-aging/anti-inflammatory protein.

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PHARMACOLOGY OF THE STR/ORT MOUSE AS A MODEL FOR OSTEOARTHRITIS: RESPONSIVENESS TO EP4 ANTAGONISTS AND AGGRECANASE INHIBITORS

R. Chiusaroli, P. Larger, C. Falcioni, A. Grotti, M. Lanza, L.P. Stasi, L.C. Rovati, G. Caselli
Rottapharm S.p.A, Monza, Italy

Purpose: The STR/ort mice develop spontaneous osteoarthritis (OA) of the knee, and are believed to be a relevant model for human knee OA. We have recently shown that treatment with either glucosamine sulfate or an MMP and aggrecanase inhibitor reduced the severity of the OA histological score as well as histomorphometric parameters in this strain. To further validate this model for drug discovery purposes, we investigated its responsiveness to further interventions within strategies currently pursued for OA treatment. We investigated in two separate studies the effects of an EP4 specific antagonist, or of a specific aggrecanase inhibitor, respectively, in STR/ort mice.
Methods: As a specific EP4 receptor antagonist we used CJ-042,794 (Murase et al., 2008). Wyeth's compound #8OO described in patent WO 2007/008994 was used as an aggrecanase1/2 specific inhibitor (ASI).
STR/ort male mice were recruited at 5 months of age (n=20-22). Compounds were administered orally once daily for 3 months, 3 and 30 mg/kg CJ-042,794, and 60 and 150 mg/kg ASI; vehicle was 0.1% methocel for both experiments. At the end of treatment, the animals were euthanized and the knee joints collected, processed for histology and blindly scored according to both Mankin's and the OARSI method. Statistical analysis was performed by the Student's t test or by ANOVA followed by Dunn's or Dunnett's tests comparing all treatment groups vs. vehicle.
For both compounds the plasma levels were monitored in limited pharmacokinetic studies in the STR/ort mouse to confirm the exposure at pharmacological relevant concentrations.
Results: After three months of daily treatment, vehicle-treated animals displayed severe OA with clefting and erosion of the articular cartilage to the subchondral bone, with prominent chondroosseous metaplasias and often inflammation and pannus. No improvement whatsoever was associated with treatment with the EP4 antagonist CJ-042,794 at either dose, at least in histopathological parameters, despite the animals were exposed to circulating levels well above its IC_{50} [approx. 10 nM] on EP4 receptors (e.g., 3 h after administration plasma concentration was 15 μg/ml [35 μM]).
Conversely, we observed a significant albeit slight decrease of OARSI score and total score following treatment with the ASI, at the higher dose (200 mg/kg). Pharmacokinetic analyses for ASI (Cmax=102 μg/mL [214 μM], $T_{1/2}$ =2h, AUC 0-24h=198 μg.h/mL), confirmed that these animals were exposed to circulating levels above the aggrecanase inhibiting concentrations [92 nM and186 nM vs. aggrecanase 1 and 2, respectively).
Conclusions: The present studies confirm the STR/ort mouse as a model of choice for evaluating OA disease modifying drugs. Moreover, they further establish aggrecanase inhibition as a disease-modifying approach, while suggest that EP4 antagonism is mainly an OA pain-controlling strategy.

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STRENUOUS RUNNING AS A MODEL FOR OSTEOARTHRITIS IN RATS: ANALYZED USING CONTRAST ENHANCED MICROCT

M. Siebelt, E. Waarsing, N. Kops, J.A. Verhaar, H. Weinans
Erasmus MC, Rotterdam, Netherlands

Purpose: Both to enhance basic knowledge of osteoarthritis (OA) development and to test the effect of disease modifying OA drugs (DMOADs), a large need for proper (small) animal models exists. Mostly, these models make use of either enzymatic or chemical intra-articular injections or surgical induced instability.
In 1998 a model for OA was described that induced OA in rats through strenuous exercise evoked by intra-cranial stimulation. This model has been modified using a (more practical) rodent treadmill with electric shock stimuli to force running for a period of six weeks. Several studies have used this model to study the effect of different DMOADs.
The goal of the current study was to investigate which early signs of OA could be detected using in-vivo contrast-enhanced microCT (CECT) in Wistar rats that were forced to run for 30 kilometres over a six week period. Second, we investigated if the induced OA was progressive after the running stimulus was stopped after six weeks.
Methods: Six male Wistar rats were trained for one week to run in a rodent treadmill machine as follows: day 1, 10 minutes at 0.60 km/hr; day 2 15 minutes at 0.72 km/hr; day 3 20 minutes at 0.90 km/hr; day 4 30 minutes at 1.08 km/hr and day 5 35 minutes at 1.20 km/hr. The following 5 weeks, the rats were forced to run for five days a week, the first 10 minutes at 0.72 km/hr in order to warm-up and the following 50 minutes at 1.20 km/hr.
All knee joints of all animals were scanned with CECT at the start of the experiment (t = 0) and directly after running at t = 1, 3, 6 weeks. After 70μl intra-articular injection with ioxaglate into their knee joint, the negative charged contrast agent (ioxaglate) enabled to measure both quantitative (volume) and qualitative (GAG-content) changes in cartilage tissue over time. At six weeks the running exercise stopped, a subsequent CECT scan was performed at 12, 18 and 24 weeks. In this abstract we present all CECT-data for condylar, patellar and trochlear cartilage up to 12 weeks follow-up.
Results: After three (p=0.02) and six weeks (p=0.01) of running significant higher attenuation values were detected, indicating GAG loss from condylar cartilage. At 12 weeks (6 weeks after running was stopped) the total volume of condylar cartilage was significant reduced, indicating OA progression (p=0.02) (Figure 1). Patellar cartilage attenuation was not

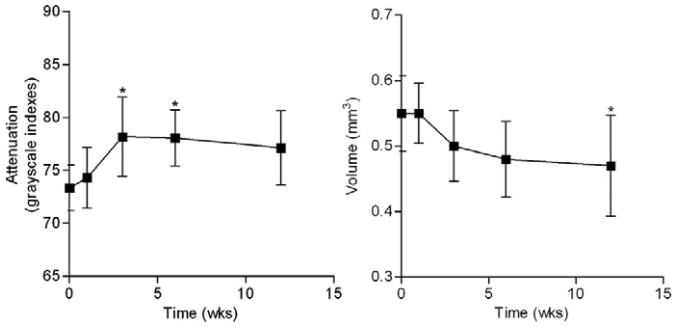

Figure 1. Condyle.

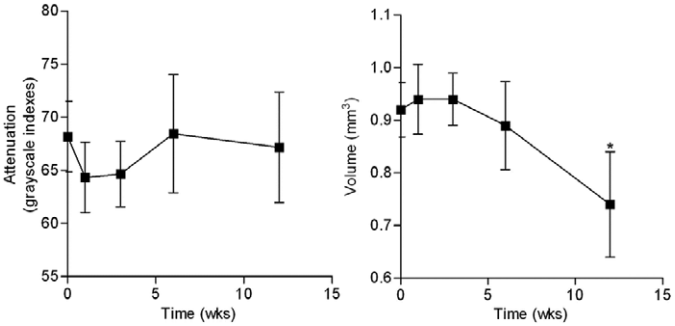

Figure 2. Patella.

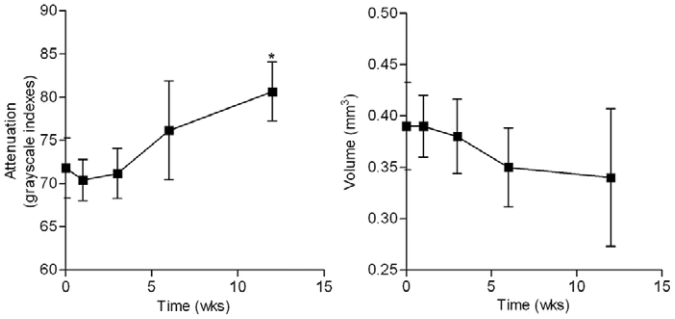

Figure 3. Trochlea.

increased, but its volume was reduced after three weeks of running, becoming significant after 12 weeks (p=0.0007) (Figure 2). Attenuation was significantly increased in trochlear cartilage at 12 weeks (p=0.009), although not significant, trochlear cartilage volume showed a clear trend of reduction from one to 12 weeks (Figure 3).

Conclusions: With CECT we successfully analyzed OA progression in knee joints of strenuous running Wistar rats. Our data suggests that 6 weeks of running damages cartilage beyond the ability for repair, which results in OA progression even when rats are not forced to run anymore. In addition, we demonstrate that besides disease progression at the weight-bearing condyles, the articular cartilage of the femoral bone and the patella is also affected. Our findings demonstrate that this model could very well be used for testing DMOADs, also after the OA inducing running stimulus has been stopped. In such a study, (regenerative) compounds can be tested without the stimulus inducing OA and counteracting a possible positive effect of the studied drug.

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TREATMENT WITH AN AGGRECANASE SPECIFIC INHIBITOR AND LUBRICIN SIGNIFICANTLY REDUCES JOINT PAIN IN A RAT MODEL OF POST-TRAUMATIC ARTHRITIS

M.A. Rivera-Bermudez, J. Tejada, T.J. Blanchet, S. Soucy, L. Savary, K. Georgiadis, C.R. Flannery, S.S. Glasson
Pfizer, Cambridge, MA

Purpose: Traumatic joint injuries lead to an increased incidence of accelerated and progressive loss of the normal structure and function of articular cartilage, resulting in pain and significant disability. The rat meniscal tear (MT) model of post-traumatic arthritis (PTA) is a primary in vivo system for testing the ability of potential therapeutics to slow cartilage degradation and pain following acute injury. It has previously been demonstrated that treatment with either an aggrecanase specific inhibitor (ASI) or recombinant lubricin is chondroprotective in the rat MT model. In the current study, we evaluated the effect of treatment with ASI and Lubricin, either dosed separately or in combination, on pain in this model.

Methods: All in vivo experimental procedures were IACUC reviewed and approved. Male Lewis rats underwent medial MT surgery on the right knee to induce joint instability. Study #1: rats were dosed with vehicle, 60 mg/kg/day ASI (PO, starting on the day of surgery), 20 μg of intra-articular (IA) recombinant lubricin (LUB1; 3X/week, starting one week post-surgery) or combined dosing with both ASI and LUB1. Dosing was terminated 5 weeks post-surgery. Study #2 replicate the dosing paradigm in study 1, but at 5 weeks post-surgery, dosing ceased and the study continued for an additional 5 weeks. The change in hind paw weight distribution between the ipsilateral knee and contra-lateral knee was measured with an incapacitance meter (Linton, CO) at various time points.

Results: Treatment with ASI and LUB1 significantly reduced pain 4 weeks after surgery. Moreover, combination therapy of ASI and LUB1 proved more efficacious than either therapy alone. Both ASI and LUB1 continued to ameliorate pain at 2 and 3 weeks after dose cessation.

Conclusions: The present study demonstrates that ASI and LUB1, both separately, and to a greater extent in combination, have the potential to significantly reduce joint injury-associated pain during the progression of PTA. Treatment with ASI and LUB1 alone also proved efficacious in reducing pain 3 weeks after dose termination, suggesting that the effect on pain can be prolonged for a substantial period after ASI and LUB1 treatment cessation. These results support the development of ASI and LUB1 as potential therapies following joint injuries in humans, to provide both pain relief as well as chondroprotection.

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USE OF GAIT ANALYSIS AS A MEASURE OF SPONTANEOUS PAIN BEHAVIOR IN MURINE MODELS OF ARTHRITIS

C. Dorman[1], **H.E. Krug**[2,1], S.R. Anderson[2], S.V. Frizelle[1], M.L. Mahowald[2,1]
[1]VA Med. Ctr., Minneapolis, MN; [2]Univ. of Minnesota, Minneapolis, MN

Purpose: Murine models of arthritis are important tools for the study of arthritis pain and discovery of new analgesic therapies, however measuring pain in mice can be challenging. We have previously described sensitive and specific methods for measuring evoked pain behaviors in mice with acute and chronic arthritis pain. But it is also desirable to incorporate the measurement of spontaneous behaviors that are sensitive to change with pain and analgesia. We have used two methods of digitized gait analysis to detect changes in spontaneous gait behavior in order to quantify pain in murine models of arthritis and to detect analgesic responses.

Methods: C57Bl6 mice were used for all experiments. In all cases arthritis was produced only in the left knee. Chronic arthritis was produced by IA injection of 10 IU Collagenase. Acute inflammatory arthritis was produced by IA injection of 10 μl 3% carrageenan. Analgesia was provided by Botulinum toxin type A (BoNT/A) (0.02 IU) which was given IA into the left knee 3 days before testing. The normal right knee served as internal nonpainful control. Mice were studied in the naïve pre-arthritic state, after induction of arthritis and after IA BoNT/A or saline control treatment. Video gait analysis was performed using DigiGait™ (Mouse Specifics, Inc, Quincy, MA) hardware and software and TreadScan digitized gait analysis system (Clever Sys, Inc. Reston, VA) Evoked pain behavior was measured by tallying fights + vocalizations/1 min in response to repeated firm palpation of the knee preformed by a single, trained examiner. Strength was observed visually and graded semiquantitatively as ability to grasp and cling to a wire grid. Visual assessment of gait was also performed and similarly graded. Student's t-test was used for statistical comparisons.

Results: No mouse appeared weak as a result of BoNT/A injection. Arthritis pain was clearly and reproducibly indicated by increased Evoked pain scores in both acute inflammatory and chronic noninflammatory arthritic mice. Analgesia after BoNT/A injection was modest in acute inflammatory arthritis and did not reach statistical significance. Anagesia after BoNT/A was statistically significant in mice with chronic arthritis as measured by Evoked pain scores. Although gross abnormalities of gait could be seen by visual observation in arthritic mice, specific alterations in gait parameters were less common, of small magnitude, and somewhat inconsistent. Similar results were obtained using two different gait analysis systems. As might be expected in a quadruped, alterations in gait due to pain did not appear to be confined to the arthritic limb, but these results were not consistent.

Evoked Pain Response - Left Knee

Mean (SEM)	Non-Arthritic	Arthritic	BoNT/A Rx	Saline Rx
Chronic Non-Inflammatory Arthritis	2.0 (0.76)	8.2 (1.22)	4.5 (1.04)	8.2 (2.39)
Acute Inflammatory Arthritis	1.3 (0.49)	12.8 (3.41)	6.1 (1.21)	10.0 (2.69)

Conclusions: Pain can be quantitated in murine models of arthritis using visual and computerized gait analysis, and evoked pain scores. Evoked pain responses increased significantly with arthritis and decreased with IA BoNT/A and appear to be reliable and reproducible. No limb weakness was noted. Although gross changes in gait can be seen in animals with arthritis, detection by digitized gait analysis is subject to individual variation, and